Method for the dissociation of double-stranded nucleic acids in a solution into single-stranded nucleic acids

Description

The invention relates to a method for the dissociation of double-stranded nucleic acids in a solution into single-stranded nucleic acids. The term nucleic acids comprises, in addition to DNA and RNA, also PNA. Further, by this term are covered natural nucleic acids, i.e. nucleic acids having full length, and fragments of nucleic acids. Fragments are parts of a nucleic acid (natural or produced by gene technology) or synthesized nucleic acids. Typical base pair numbers of fragments are for instance in the range of smaller than 10 to more than 2,000.

Methods of the above type are for instance needed in the field of amplification or multiplication of nucleic acid structures or for the hybridization of nucleic acids to be investigated with known nucleic acids for the purpose of obtaining information about the sequence of the un-known nucleic acids (key words: DNA hybridization techniques, DNA chips). The basis for many gene-technological analysis methods is the selectively operating PCR (polymerase chain reaction), a simple multiplication method for specifically selectable DNA's or DNA fragments in a reaction chamber (see also US-A-4,683, 202) or in situ, for instance in a tissue cut. After the introduction of temperature-stabilized ploymerases, this method is now widely used in gene-technological laboratories.

PCR is in particular the specific multiplication of a relatively short segment of the two-stranded DNA in simple temperature cycles, this segment being precisely selected from the genome. The selection of the DNA segment is achieved by a so-called pair of primers, two DNA pieces of a length of approximately 20 bases each coding the both-side ends of the selected DNA segments. Multiplication is

achieved by an enzyme with the name polymerase. The PCR reaction takes place in an aqueous solution, where a few molecules of the original DNA and sufficient quantities of polymerase, primers, tri-phosphates of the four nucleotides, activators, and stabilizers are present. In each temperature cycle, first the double helix of the DNA is "molten" at approx. 95°C, with both strands being separated from each other. At approx. 45°C then the primers are added on in a precisely fitting nucleotide sequence of the individual DNA strands ("hybridization"). At 72°C, the double helix is complemented again by the addition of the complementary nucleotides to a growth end of the primers by a particularly temperature-resistant polymerase (taq polymerase), so to form a new double helix again. Thereby, the selected DNA segment between the primers is basically doubled. In 30 cycles, thus, round about 1 billion DNA segments are produced from a single double-strand of the DNA as the original material, both ends of said segments being identical with the primers.

Multiplication of the DNA takes place, in today's PCR devices, relatively quickly compared to previously used cloning methods in living cells. With an optimized control program, an approx. 400 base pairs long DNA fragment can be amplified from decomposed cell material within three hours by using a conventional PCR device, so that the DNA concentration will be increased to clearly above the detection limit (in the agarose gel under UV irradiation and staining with ethidium bromide).

Melting of the DNA has previously been performed at a temperature slightly above the empirically determined melting temperature. Investigations have shown that heating-up to this temperature for half a second will be sufficient for a complete decomposition of all double-helix structures. The hybridization will not take long, too, if the primers are present in sufficient concentration. At an optimum concentration, one to two seconds will suffice. The completion of the DNA has a very high speed: under opti-

mum temperature and concentration conditions, 50 to 100 bases per second can be added by a special polymerase (C. R. Newton and A. Graham: PCR; Spektrum Akademischer Verlag; Heidelberg, Berlin, Oxford, 1994; page 31). Since in general only segments of up to 400 bases length are needed for the analyses, ten seconds are fully sufficient for the extension).

The time duration of a temperature cycle depends thus to a large extent on the heating-up and cooling-down speed, this in turn depending on the liquid volume, on the dimensions of the vessel, and on the heat conductivity of the vessel walls and of the reaction liquid. There are only needed a few seconds for each temperature stage, in part even less.

It is well known in the art to keep the transition times to the following temperature level as short as possible by reducing the sample volume, increasing the specific surface and by pneumatically applying a cooling block (DE 197 17 085 A 1). This is however only possible at appreciable mechanical and microsystem-technical expenses.

From document WO 98/00562 it is known to perform the melting process by means of an electrical field being switched-off or inverted in the course of the reaction.

From document WO 96/41864 it is known to adjust the temperature in the solution required for melting by means of irradiation of IR and UV light.

From document WO 98/06876 it is known to perform the heating-up process for melting by that a solid-body substrate present in the solution is heated up by means of irradiation of electro-magnetic waves. The employed electro-magnetic waves have frequencies below 1.07 GHz.

A high PCR speed is desirable in particular for the following reasons. Within the framework of the "Human Genome Project", it is tried to de-code the sequence of the human genome. Since this genome has a size of approx. 3 billion base pairs, and up to now (in October 1998) only

6.8 % thereof have been sequentiated, latest forecasts state that there will be another 25 years needed to terminate the project. The aid programs for this project assume, however, that already in six years all bases should be sequentiated. Since the PCR is a substantial component of the sequentiation and all other steps of the sequentiation are much quicker today, the success of the project will depend in particular on the PCR speed. Further, there are PCR applications that can be used for the detection of certain tumor cells in human tissue. Should such analyses be performed during an operation at open patients, a maximum analysis duration of 10 minutes is required. The up to now fastest described PCR devices (A. T. Woodley et al., "Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device", Anal. Chem. 68, 4081, December 1996) will need however at least 15 minutes for a program with 30 cycles. The time required for the decomposition of the cells and the release of the DNA is not included herein. Finally, PCR is for instance also used for the selection of aptamers being affine against given destination substances or ribozymes from highly complex DNA libraries (10^{15} and more).

A further problem with the known PCR methods is the irreversible destruction of the polymerase by the high temperature during melting. Although there are available today polymerases being well optimized with regard to temperature stability, the efficiency of the enzymes will considerably drop at each cycle. The half-value time of the activity of usual polymerases (of *Thermus aquaticus*) at 95°C is 40 minutes (C. R. Newton and A. Graham: PCR; Spektrum Akademischer Verlag; Heidelberg, Berlin, Oxford, 1994; page 31). Especially during the last cycles, however, a very high activity of the polymerase is required, since very many DNA amplificates have already been produced, all of which are however to be extended. Therefore, a correspondingly larger amount of the expensive enzyme has to be employed. Some PCR methods solve this problem by a suc-

cessive extension of the elongation times in each cycle. A polymerase molecule can then be used several times successively at different locations during a cycle. This will however increase the total duration of the PCR. It is desirable, if an optimization of the extension speed of the polymerases could be possible, rather than having to further improve the temperature stability of the enzymes.

It is common to prior art technologies that the demand of electrical energy because of the necessary heating and cooling processes is relatively high, with the consequence that corresponding power supply units have to be provided. This makes the devices of the state of the art voluminous and heavy and makes on-site application outside a specially fitted laboratory difficult.

Therefore, the invention is based on the technical problem to provide a method, by means of which within shortest time a double-stranded nucleic acid can be split up into single strands. In particular it is desirable to enhance the cycle time for the PCR amplification of nucleic acids and to reduce it to a few seconds. It is further desirable to avoid the damaging heating-up to high temperatures of the polymerase, and to thus make the PCR more effective and simultaneously save expensive polymerase enzyme. Finally, a reduction of the electrical power consumption for the purpose of mobile use, for instance with batteries, would be desirable.

For achieving this object, the invention teaches that the decomposition of the nucleic acids into single strands is performed by irradiation of electro-magnetic waves, the frequency and intensity of said electro-magnetic waves being selected such that the hydrogen bridges between nucleotides of a double-stranded nucleic acid being released by immediate interaction of the electro-magnetic radiation with the double-stranded nucleic acid.

The invention is founded on the following. The exact structure, the binding lengths, the binding angles and the

binding energy of the hydrogen bridges between the nucleic acid and the DNA strands and the molecular mass ratios of the nucleotides are very precisely known. If then the frequencies of the electro-magnetic waves are selected such that the waves show an as low as possible interaction with the medium, i.e. the reaction buffer or its main components, an (undesired) thermal heating-up of the total mixture can be avoided. It is understood that the frequencies further should be selected such that no disturbing absorption by the polymerases will take place.

For melting the nucleic acid, energy has to be supplied to the molecule, the amount of which is at least as large as the binding energy of the hydrogen bridge. This takes place in the previously described PCR by a temperature rise. The essential discovery of the invention is that the nucleic acid molecules can be excited to vibrations by electro-magnetic alternating fields, due to the inhomogeneous distribution of charges. The resonance frequencies can be determined by absorption-spectroscopic measurements of dissolved nucleic acids in the millimeter-wave and submillimeter-wave range (10 - 500 GHz) or in the far IR (0.5 - 10 THz). Some of these vibration states cause a radial periodic valence vibration between nucleotides of different strands linked to each other by hydrogen bridge bonds. If energy is irradiated in the range of these resonance frequencies by means of a high-frequency resonator of sufficient power, this may lead to a release of the hydrogen bridge bonds and thus a separation of the two strands of a double-stranded nucleic acid. The millimeter-wave induced separation into the individual strands can be proven by the hyperchrome effect of the nucleic acids by means of a measurement of the UV absorption or transmission (wavelength 260 nm). When the high-frequency resonator is switched off (or when the nucleic acid is transported into an area of low electrical field), the single-stranded nucleic will automatically rehybridize to double-stranded nucleic acid. It is thus possible to selectively effect, by specific irradiation of electro-

magnetic waves of a defined frequency, in particular nucleic acid resonance frequencies being far enough away from resonances of the medium or of the polymerases, a separation of the nucleic acid strands from each other, and that without an undesired heating-up of the buffer and in particular of the polymerases, as it can be found in prior art methods. This is proven (beside the not observed heating-up) by that the separation requires a few milliwatts only, for instance 0.1 to 1,000 milliwatts, in particular 1 to 100 milliwatts, with suitable design of the exposition device and positioning of the reaction vessel.

In this context it has to be noted, further, that the processes of the separation of the double-stranded nucleic acid need not be limited to the direct separation of the hydrogen bridge bonds. Vibration states of the total molecule or of parts thereof may also play a role, since these vibration states will at any case transfer the hydrogen bridge bonds into excited states, if the vibration states lead to for instance sterical tensions in the range of the hydrogen bridge bonds. It is therefore also possible that the excitation of vibration states of the strands may lead to a separation of a double strand. Therefore the term immediate interaction of the radiation with the double-stranded nucleic acid means, in addition to excitations of the hydrogen bridge bonds, also the excitation of other bonds of the nucleic acid at least leading to one excitation, i.e. weakening of the binding forces. Excitation substantially means the excitation of elongation and/or flexural vibrations of molecular bonds. In the case of vibration of the molecule structure of one or both strands, superimposed structures may also be formed, such as wave phenomena and shearing forces.

Since in a nucleic acid there is always at least one bound nitrogen atom involved, the hydrogen bridges have at any case always a lower binding force than that of the surrounding water, which has only stronger-bound oxygen atoms as donors. It is thus possible, with a suitable selection of the irradiation frequency, to specifically melt the nu-

cleic acid, without simultaneously heating the surrounding water to a disturbing degree. But frequencies above the resonance frequency of water can also be used, since for instance in the range of 0.5 to 2.0 THz characteristic resonances of the molecule structure can be observed ("breathing"). When selecting such high frequencies (adjusted to a suitable resonance frequency of the molecule structure), a decomposition of the hydrogen bridge bonds takes finally place because of the generated "tensions" in the area of the hydrogen bridges. It is also possible to irradiate in the range of the hydrogen bridge resonances as well as in the range of the structure resonances.

It is preferred, in the framework of the invention, to adjust the frequency of the electro-magnetic radiation in the range of 10 GHz to 2 THz, preferably 10 to 150 GHz, most preferably 30 to 79 GHz, for instance 45 to 53 GHz, such that the adjusted frequency excites at least partly such vibration states of the nucleic acid resulting in a separation solely of the hydrogen bridge bonds, not however separating bonds of a polymerase (this includes slight decomposition rates of up to 10 %, preferably less than 2 %, of the rate at heating-up to 95°C) or leading to a temperature increase of a medium (this includes insubstantial temperature increases of a not thermostated medium of up to 20°C/min, preferably of less than 5°C/min). This is the frequency range where experimentally suitable frequencies have been found, in order to melt double-stranded DNA at temperatures below the DNA melting temperature, even down to 20°C.

Of an independent importance within the framework of the invention is the utilization of the method according to the invention in a method for the in-vitro amplification of nucleic acids with the following steps: a) decomposition of the nucleic acids into single strands, b) hybridization of primers to the single strands of step a), elongation of the hybridized primers of step b) by (desoxy-) ribonucleoside triphosphates by means of a polymerase, d) return of the

nucleic acids obtained in step c) into step a), steps a) to d) being repeated so often, until a given amplification factor has been achieved and the electro-magnetic radiation being irradiated in step a). It is preferred to use polymerases being optimized to a nucleic acid synthesis speed in step b). These need not have anymore the temperature resistance as required for the state of the art.

Subject matter of the invention is further a device for carrying-out a method according to one of claims 1 to 4, comprising a reaction chamber for receiving a solution with nucleic acids, a device for generating electro-magnetic waves and an antenna element for irradiating the electro-magnetic radiation, the antenna element being arranged immediately at the reaction chamber and at least one operating frequency of the device for generating electro-magnetic radiation being in the range of 10 to 250 GHz, preferably 30 to 79 GHz, and/or 0.5 to 2.0 THz. The term antenna element designates any components being capable to emit electro-magnetic alternating fields. These may for instance be arranged around the reaction chamber or outside at one side only. Imaginable is also the integration of an antenna element in the interior if the reaction chamber. A particularly advantageous embodiment for the lower frequency range is characterized by that the reaction chamber is disposed within a resonance body operating as an antenna element, in particular a cavity resonator or a waveguide.

The reaction chamber may be configured in various ways. The reaction chamber may for instance be an agitator vessel reactor. This means that the reaction solution remains stationary or possibly is agitated. It is however also possible to adapt the reaction chamber as a tube reactor, in the interior of the tube reactor and in the direction of longitudinal extension of the tube reactor standing electro-magnetic waves being generated. In a tube reactor, the reaction solution is mobile and flows in the direction of the longitudinal extension of the reactor. The reaction progress or the reaction stages are a function of the position (in a

first approximation one-dimensional along the longitudinal extension). Another preferred embodiment of the reaction chamber is a capillary (possibly closed on both ends) being aligned with regard to its longitudinal extension at least in part in the direction of the electro-magnetic field vector of the electro-magnetic radiation.

It is achieved, by the invention, that the heating-up and cooling-down stages are not needed for the PCR, so that the cycle time and thus the total duration of the amplification process is substantially reduced, compared to the prior art PCR. Simultaneously the polymerase needed for the multiplication is practically not damaged by heat denaturation, so that a smaller amount of the expensive enzyme may be used. This basically relies on that the resonances of the polymerase are typically not at the same frequencies as the resonances between two nucleic acid strands or molecule resonances in one strand. It is important at last that the melting-on process is performed, according to the invention, not by temperature increase, but by irradiation of electro-magnetic waves with a defined nucleic acid or hydrogen bridge bond specific frequency.

In the following, details of the invention and other possible embodiments of the invention will be explained.

For the PCR, the reaction mixture passes through the following physical and chemical state modifications:

a) The reaction mixture is irradiated with electromagnetic waves of one or several defined frequencies of sufficient power. Double-stranded DNA is transformed thereby into single-stranded DNA. The temperature of the reaction mixture is not essentially affected by the electromagnetic vibrations.

b) The irradiation of the electro-magnetic waves is terminated, and the reaction mixture is set to a suitable temperature below the addition temperature of the primers. The

primers can add to the complementary locations of the single-stranded DNA.

c) The reaction mixture is set to a temperature where the polymerase will fill up the complementary strand at an optimum speed with nucleotides. These three steps may be repeated so often as desired. The resulting double-stranded reaction product is used in (a) again.

In practical applications it is possible to adjust the same temperature, for instance in the range of 20°C to 80°C, for steps b) and c). The total reaction is then isothermal. This permits a considerable simplification of the devices compared to PCR of the state of the art, where at high precision and consequently high technical expenses different temperature stages have to be adjusted and controlled, and this as fast as possible. In other words, with an isothermal proceeding according to the invention, also the time-consuming heating-up and cooling-down phases between the various steps are not needed.

The reaction mixture is composed of the usual components, as they are used for the conventional PCR or the improvements thereof. These are at least one suitable solvent, a very small amount of nucleic acid as a matrix, one or more suitable oligo nucleotides as primers, desoxy nucleotide triphosphates of all nucleic bases in the matrix between the primers, a substance catalyzing the chain extension (in the further text called polymerase).

In principle, all variants, modifications or improvements of the PCR (in particular the sequentiation reaction with the chain interruption method according to Sanger) may be performed in this method. If the case arises, corresponding adaptations can be performed, so as to benefit from the advantages of the invention in an optimum manner.

The most important benefit of the invention is that in practical applications the temperature of the reaction mixture will never reach the melting temperature of the in-

volved nucleic acid pairs, the less will exceed it. Usually a PCR can be performed at temperatures of not more than 75°C, in particular not more than 60°C. Consequently no special heat-resistant polymerases are needed anymore. It is obvious to select or construct the employed polymerases such that they will synthesize as fast as possible the elongation of the DNA strand to be newly built up. So the PCR step having been up to now the slowest one and determining the overall speed is clearly shortened. Of course, also the heating-up and cooling-down times for the separation of the two DNA strands are saved, since the electro-magnetic waves act immediately on the DNA. In the conventional PCR first the heating block and then the reaction vessel and the surrounding solvent have to be heated up, before heat will be passed on to the DNA molecules.

In practical applications the electro-magnetic waves will be generated by an electronic high-frequency resonator and are directed with an irradiation device (antenna) to the closely positioned reaction mixture. As a high-frequency resonator for instance Gunn, IMPATT, backward-wave or clystron oscillators can be used. A (fundamental) frequency of a HF generator being too low may be increased to the required level by frequency multiplication for instance at a varactor diode. It is however also possible to arrange the reaction mixture within an electrical resonance body (for instance a cavity resonator or a waveguide) being excited to standing vibrations by the generator. Further it is possible to use an oblong thin reaction vessel (e.g. a capillary) being passed by the reaction mixture at low flow speed. Along the axis of this reaction vessel a standing wave is generated, forming nodes at regular distances, where the intensity of the electro-magnetic waves is concentrated, whilst there are areas shifted in phase by 90° where the waves extinguish themselves completely. The reaction mixture is regularly molten on during the passage of such positions. By suitable selection of the parameters (linear flow speed in relation to the wavelength) this can be syn-

chronized with the cycles of the PCR. Thus results a continuous amplification along the reaction vessel. The millimeter wave components as well as the capillary structures as configured reaction chambers can easily be produced by microsystem technologies, thus an integration of the components on one module being specially enhanced.

The frequency of the electro-magnetic waves results, as per the above, from the radial resonance vibrations between the nucleotide strands. Normally the frequency is between 30 and approx. 60 GHz. It may be reasonable to irradiate at several frequencies in this range at the same time. The (additional) irradiation at completely different frequencies may be reasonable under certain circumstances, in order to dissolve cross links at complicated tertiary structures of the DNA and thus to facilitate the amplification of the DNA section. For an optimum function, the polymerase normally requires a temperature above room temperature (for the today usual taq polymerases approx. 72°C). By measured-out (additional) irradiation at a resonance frequency of the solvent (normally a salt-bearing aqueous buffer) the temperature of the reaction mixture may be increased. Thus, further, an additional heat source is not required.

The invention can further be used in an advantageous manner for the in-situ PCR. A tissue cut is for instance treated with PCR reagents, with at least one primer carrying a marking. Marking means any kind of marking atoms or marking molecules being effective with regard to measurement or color. Then the tissue cut is exposed to the above reaction cycle with the result that nucleic acids can be detected at a high efficiency and lateral resolution. It is achieved, by the invention, that desiccation of a tissue cut and/or denaturation of substances therein can be prevented, due to the smaller heating-up, and that under prevention of the measures otherwise required.

In the following, the invention will be described in more detail, based on figures representing examples of embodiments only. There are:

Fig. 1a a representation of a apparatus for the determination of frequencies for the separation of the nucleic acid strands of a double-stranded nucleic acid,

Fig. 1b a representation of an apparatus for the observation of the separation of the nucleic acid strands by means of UV transmission,

Fig. 1c a diagrammatical representation of the geometrical fundamentals for a device according to the invention,

Fig. 1d an apparatus for carrying-out the method according to the invention,

Fig. 1e an embodiment alternative to the subject matter of Fig. 1d,

Fig. 1f an embodiment alternative to the subject matters of Figs. 1d and 1e,

Fig. 2 attenuation measurements of buffer and NaCl solution above water,

Fig. 3 attenuation measurements of various high-molecular DNA's above buffer,

Fig. 4 attenuation measurements of various short DNA's above buffer,

Fig. 5 the concentration dependence of the attenuation above buffer for double-stranded DNA's,

Fig. 6 the time dependence of the UV absorption at a UV frequency specific for double-stranded DNA at millimeter waves irradiation for the observation of the hypochromatic effect,

Fig. 7 a representation according to Fig. 6 with recombination after millimeter wave cut-off, and

Fig. 8 a representation according to Fig. 7, however with additional of further double-stranded DNA during the millimeter wave irradiation at the time t = 300 s.

In Fig. 1a can be seen an enclosure 1, where a waveguide 2 (80 mm long piece WR28 waveguide with standard flanges for the Ka band, WR19 waveguide for the U band) is provided. Waveguide 2 is excited by means of a HF generator to an adjustable frequency. For this purpose a transmitter antenna is disposed in the frame of waveguide 2. Further, a receiver antenna is provided being connected - same as the transmitter antenna - to a spectrum analyzer 9. A capillary 4 from glass (length 80 mm, internal diameter 0.7 mm, external diameter 1.6 mm) extends through the interior of waveguide 2, through this capillary liquid being sucked by a suction device 8 from a storage vessel 10 and adjusted for instance to a liquid level of 10 mm above the upper edge of waveguide 2 (vertical arrangement of capillary). With this structure, the attenuation measurements described in the following have been performed, at frequencies in the range of 40 to 60 GHz.

In Fig. 1b is shown an apparatus for observing the hypochromatic effect. The essential elements are a waveguide 2, a HF generator 3 adjusted to a suitable frequency and comprising a transmitter antenna, and a capillary 4 (with the liquid to be examined). In the frame of waveguide 2 is provided an adaptation element 7 for the adaptation to the irradiated high frequency. Further a UV light source 5 (200 to 1',000 nm) and a UV detector 6 (spectral selectivity in the wavelength range of 250 to 260 nm) are provided, the arrangement having been made such that the capillary will be irradiated in its longitudinal direction. The UV transmission measurements have been made at 260 nm and a HF frequency of 49 GHz. By an arrangement according to Fig. 1b, 50 % of all employed double-stranded nucleic acid molecules can be decomposed within 30 s at a transmission power of 20 milliwatts

Fig. 1c shows a suitable orientation of capillary 4 and waveguide 2 or electrical field vector with regard to each other for a closed reactor. It can be seen that the longitudinal extension of the capillaries should be parallel to the vector of the electrical field of the (standing) electromagnetic wave.

In Fig. 1d is shown a simple embodiment of an apparatus suitable for PCR. The essential elements are waveguide 2, adaptation element 7 and capillary 4. Capillary 4 is closed on both ends. Generation of the high frequency is achieved here by a Gunn diode 11.

In Fig. 1e is shown an arrangement by means of which reactions in a multitude of capillaries 4 can be performed. It has to be noted that each capillary is disposed in a maximum of the electrical field vector of the standing wave, as it is shown schematically by the indicated wave.

Fig. 1f shows an embodiment of independent inventive importance. Here a reaction section of capillary 4 is arranged in the longitudinal direction of waveguide 2 and orthogonally to the electrical field vector and in the direction of the standing wave. The reaction mixture passes through capillary 4 with a defined flow speed. During the passage, separation of the double-stranded nucleic acid into single strands will take place at positions of high electrical field strength, whereas in sections of low electrical field strength the hybridization and polymerase-catalytic addition of nucleotides will occur. During the passage, thus, a multitude of amplification cycles are acting, the number of cycles being given by $n = 2l / \lambda$ (n = number, l = length of the capillary in wave direction, λ = wavelength). The flow speed can easily be optimized in conjunction with the temperature (held constant) with regard to maximum total amplification.

In the attenuation measurements performed with the apparatus of Fig. 1a, first the attenuation, i.e. absorption as a function of the frequency, of the medium compared to water

has been determined. These attenuation curves have then been used as references for the determination of the attenuation when a DNA is brought into the medium. In this way a millimeter wave absorption spectrum is obtained, freed from medium artifacts.

In the UV absorption measurements, the selected UV wavelength is specific for single-stranded DNA (hyperchromatic effect). A high transmission means thus a low concentration of single-stranded DNA. Low transmission however indicates the presence of single-stranded DNA.

In Fig. 2 can be seen the attenuation of a buffer of the composition 9 g/l NaCl, 50 mM Tris/HCl, pH 8, in 11 ddH_2O and of 1 M NaCl/dH_2O (d means distilled). Whereas for the NaCl solution a strong minimum is observed at approx. 45 GHz, the spectral distribution of the buffer is nearly horizontal. The buffer or the NaCl have been used in all following experiments in an unchanged condition.

In Fig. 3 are shown the attenuations of various high-molecular DNA's (for break-down of all DNA samples used here and in the following see Tab. 1). It can be seen that at least for relatively short DNA a characteristic minimum occurs in the range from 47 to 51 GHz; most probably a frequency where the DNA molecules are at least partly immediately excited. Fig. 4 confirms this in particular by the comparison of the spectra of double-stranded DNA with the same, but single-stranded DNA. Fig. 5 proves the concentration dependence for the double-stranded DNA 1.

In the experiments of Figs. 6 to 8, at t = 0 a solution of the double-stranded DNA 1 of the manufacturer Sigma with a concentration of 10 mM has been used. In the lower section of the diagram, the operating voltage of HF generator 3 is shown. Operating voltage 0 means HF generator 3 is switched off. Operating voltage 5 V means HF generator 3 is switched on.

In Fig. 6 can be seen, at small times and switched-off HF generator, a high and constant transmission due to the presence of double-stranded DNA. At the same time when HF generator 3 is switched on, transmission will however drop, caused by the reduction of double-stranded DNA concentration. The "peaks" are due to emission artifacts of the UV source.

In the experiment of Fig. 7 has been verified that the concentration drop of double-stranded DNA is really correlated with the formation of single-stranded DNA. After switching the HF generator off, it can be seen that the transmission rises again; the result of the recombination of the individual strands to double-stranded DNA.

In the experiment of Fig. 8, further double-stranded DNA has been added during the switch-on time of HF generator 3, with the result that intermediately the transmission rises again.

A device for carrying-out the in-vitro PCR method according to the invention is in principle designed as those of the state of the art. The essential difference is that in addition to or in lieu of the conventional devices for heating-up to 95°C, a device for the irradiation of electro-magnetic waves into the reaction chamber is provided. With regard to details thereof, reference is made to Figs. 1a to 1f; the further components correspond to conventional construction and need not be explained in more detail. It is understood that UV source and UV detector are not needed for carrying-out the PCR.

Sample	Description	Conc.	Literature	Manufacturer
DNA1	10 b long oligo nucleotide with the sequence 5'-GCGAATTCGC-3'; is definitely present in 1 M NaCl solution in a double-stranded form, since it has a palindromic sequence. Melting temperature approx. 50°C; synthesis scale: 2 µmole.	0.4 g/l	[Bresslauer 1986]	TIB Molbiol
dsDNA1	Identical with DNA1, however different manufacturer and 10 µmole synthesis scale.	2.0 g/l	[Bresslauer 1986]	Interactiva
ssDNA2	10 b long oligo nucleotide with the sequence 5'-GGGAAAAGGG-3'; is present in 1 M NaCl solution in a single-stranded form, since it cannot form base pairs with itself. Calculated melting temperature (for a pairing with a complementary nucleotide) is however as high as for DNA1 and dsDNA1 (approx. 50°C); synthesis scale: 10 µmole.	1.9 g/l	Own design	Interactiva
pUC8	2.5 kb circular plasmide. Is present in the so-called supercoiled form. Is normally used for cloning E.coli types.	0.1 g/l	[Edwards 1984]	Sigma
L_gtll	Modified DNA of the lambda prophagene, type gt11. Approx. 50 kb long and linear; normally used for cloning long DNA fragments (up to 10 kb) in special E. coli types.	0.05 g/l	[Sanger 1982]	Promega
Lambda	Cleaned-up DNA of the lambda prophagene. In higher concentration than L_gt11 and not modified. Approx. 50 kb long.	10 g/l	[Sambrook 1989]	Sigma
HSP	Vertebrate DNA cleaned-up from herring sperm; heterogeneously sheared DNA. Economic and available in high concentration.	10 g/l	[Sambrook 1989]	Fluka

Drawings

Legend:

Fig. 1c
Position and orientation of the capillary
E field vector
Propagation direction of
electro-magnetic wave
H field
vector

Fig. 2
Solvent
dB above ddH_2O

Fig. 3
High-molecular DNA
dB above buffer

Fig. 4
Oligos
dB above buffer

Fig. 5
Concentration dependence at 49 GHz
dB above buffer

Figs. 6, 7, 8
Transmission